FAX COVER SHEET

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TO: Mr Jack Benadetta (I apologize for the spelling)

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TOTAL NUMBER OF PAGES: 7
(Including Cover Sheet)

MESSAGE: I apologize if some of the enclosed material is a "rehash" of what you already know - It is done for completeness and gives you the background for the development of the new product.

May Jacobson and I will be meeting with Kevin at 800 Huntington on Monday - I will let you know how to reach us

Sherwin

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To Jack B
From Dr SVK
cc MSJ PhD

Prior to presenting some of the information you might need for "European Submission" - some background might be helpful.

① The new material is a coagulant in that it mimics the action of thrombin. It can convert a pure saline suspension of Fibrinogen* We are in the process of further characterizing the material (site of action shown in the enclosed (figure 1- fibrinogen→Fibrin).

* TO FIBRIN, SUGGESTING THROMBIN IS PRESENT.

② The material presently used is a procoagulant. We have previously demonstrated at CBRL that the active component is Xa (Factor Xa). It initiates the coagulation pathways as shown in figure 1. This material can not* convert fibrinogen to fibrin.

* DIRECTLY

3. The mannitol that is combined with the ACD anticoagulant is well known to european regulatory agencies. It is a component of two red cell preservative solutions SAGM and ADSOL. The mannitol is used in those preservative solutions to decrease micro vessicle shedding. It serves a similar purpose in our procedure and facilitates the separation of the precipitated proteins and red blood cells from the supernatant following centrifugation. The supernatant contains the coagulant.

4. We have used the SmartPReP to prepare the coagulant. The tubes we used to prepare the coagulant were of the same material but shorter in length to accommodate the internal dimensions of the SmartPReP. Most of our studies with the proposed test tubes were done using stand alone centrifuges using the same g force and time that we will have in both SmartPReP systems.

Preparation of the Coagulant

The coagulant is prepared from a whole blood sample that utilizes a modification of the fractionation procedure based on *Cohn's Method 6. All other procedures utilize plasma as the starting material and in most instances use cryoprecipitation as the first step in plasma fractionation.

*EDWIN

Utilizing a proprietary level of ethanol at a pH of 6.5 - 7.2 with a specified incubation time, the mixture is centrifuged. During this process the red blood cells and proteins such as fibrinogen, proteins C and S and antithrombin III are precipitated. The precipitated proteins and red cells are separated from that in solution by a conventional liquid-solid separation technique such as centrifugation.

We have characterized to some extent the proteins removed and the content of the supernatant which is our coagulant. Additional

Studies are on the drawing board which will: (1) further characterize the coagulant and its relationship to thrombin; (2) characterize what proteins are contained in the precipitate and their quantification; (3) Determine clotting times with both platelet concentrates (various levels) and platelet-poor-plasma using the existing 3:1 ratio and possibly others; (4) Evaluate the growth factor release over time when the coagulant is combined with a platelet concentrate; (5) prepare the coagulant on the Ben-2 Smart PReP using the proposed test tubes and disposables.

Existing Characterization of the Coagulant

① Clotting Studies

Ratio Platelet Concentrate : Coagulant	Clotting Time - Seconds			
	Initial	1 hour	3 hrs	5 hrs
3 : 1	7.5 ± 1.5	8.8 ± 0.7	9.4 ± 1.2	10.5 ± 1.8

n = 18

(2) Comparison of Coagulant to Normal Plasma

Antithrombin III Reduction	84.5% - n=22
Fibrinogen Reduction	100% - n=16
Protein C Reduction	41.5% - n=5
Factor XIII Reduction	77.1% - n=5
Plasminogen Reduction	43.0% - n=5
Prothrombin Reduction	5.0% - n=2

Coagulant Preparation Scheme

8mL Whole Blood – ACD- Mannitol

1.6mL Add* 95% Ethanol and 0.1mL 10% $CaCl_2$ = 1.7mL TOTAL

Incubate the mixture - 30 min.

Centrifuge in the SmartPREP

Separate Supernatant from Precipitate by Serum Separator Device

Precipitate
RBC's
Fibrinogen
Other Proteins

Supernatant
Coagulant
"Albumin"
"Prothrombin" ↗ ?? I BELIEVE HE MEANT "THROMBIN"